THE November–December number of the Hungarian entomological journal—*Rovartani Lapok*—is a jubilee number in honor of Alex. Mocsary, for his forty years' service in the Hungarian National Museum. Most useful is a list of his numerous publications. A list of species (65 in all) that have been named in his honor is given, to which various friends add new species in all orders in this number of the journal.

MR. C. C. GHOSH has published an account of the life-history of a neuropterid fly—*Croce filipennis.*[4] These delicate insects are but little known, and a figure of Savigny had long done duty as the only known larva of the family. The larva of *Croce* is very similar to that of Savigny, with a large *Chrysopa*-like head and jaws and an extremely slender prothorax; the abdomen broad and flat. They live in houses in India, and feed on silver-fish and bed-bugs. The pupa is formed within a spherical cocoon; the larval stages last for nearly a year, and the adult appears for only a few days in April.

DR. E. MJOBERG is the author of a long article on morphology and classification of the biting and sucking lice.[5] A number of species are described as new, mostly from the old world, and several new genera. One, *Neohæmatopinus,* is made for *Hæmatopinus sciuropteri* Osborn. He concludes that both Anoplura and Mallophaga should be included with the Psocidæ in the Corrodentia, as three subequal groups; the Anoplura more closely related to the Mallophaga than either to the Psocidæ. A useful bibliography is appended.

CARL HENRICH has published a large paper on German plant-lice which will be of use to our students of these insects.[6] He divides the family into the usual six tribes, but appears to be unfamiliar with some of the recent generic changes.

DR. N. J. KUSNEZOV brings up cases of probable viviparity in certain pierid butterflies of northern Russia.[7] In examining the anatomy of certain pierids (*Colias*) he found fully developed larvæ in the lower part of the oviduct, and no chlorion around them. These larvæ were bent double, with the head toward the aperture. He therefore concludes that at least sometimes the larvæ are born alive, or at least so far advanced that the eggs hatch very soon after deposition. The reason for this intrauterine development of the embryo he believes is the short season in the northern localities. Two species of Tineids have been recorded as viviparous.

NATHAN BANKS

SPECIAL ARTICLES

PROTECTIVE ENZYMES[1]

IN this preliminary paper the authors will bring together the results which thus far show some important relations and reactions carried out by certain protective enzymes of fruits. This work originated in the efforts of one of us (Cook) to determine the toxicity of tannin. It is well known that tannin is one of the most abundant of plant products, and it has been repeatedly stated that it occurs in green fruits. Although the work referred to above gave very definite results on the toxicity of tannin, it became evident that there was some factor or factors in the living fruit which had not been taken into consideration, making it necessary to attack the problem from the biochemical standpoint.

Pomaceous fruits were most satisfactory for our purpose, although the fruit of the tomato and other plants were also used. As the work progressed many difficulties presented themselves, such as the uncertain and more or less unreliable methods for quantitative determination of tannin.

[4] *Journ. Bombay Nat. Hist. Soc.,* 1910, p. 530.

[5] "Studien über Mallophagen und Anopluren," *Arkiv f. Zoologi,* Vol. VI., No. 13, 296 pp., 5 pls., 1910.

[6] "Die Blattläuse, Aphididæ, der Umgebung von Hermannstadt, mit einem Index und Figurenerklarung," *Verh. Mitt. Siebenb. Ver. f. Naturwissensch. zu Hermannstadt,* LIX., pp. 104, 1 pl., 1910.

[7] "On the Probable Viviparity in some Danaid, *i. e.,* Pierid Butterflies," *Hor. Soc. Ent. Ross.,* XXXIX., pp. 634–651, 1 pl., 1910.

[1] By permission of the Delaware Agricultural Experiment Station.

Among other things brought to light by these studies was the very great variability in the amount of tannin at different times and under different conditions. It is well known that tannin occurs in great abundance in certain tissues of the plant and in injured parts, and these facts led to original studies on the possible function of tannin in plants. However, we did not expect to find the great variation in amount of tannin dependent upon the length of time between the removal of the fruit from the tree and the analysis of this same fruit, for it was finally learned that there was a rapid increase in the amount of tannin or like bodies in the normal fruit immediately after removal from the plant, and that the tannin continued to increase in quantity for some time. Although the greatest increase was, as previously stated, immediately after removal from the plant.

For instance, a sample was taken by dropping the fruit into boiling water immediately after plucking to stop all enzyme action, and the tannin determined at once. At the same time another sample of the fruit on the same tree was injured by repeated puncturing of the stem and fruit with a pin and allowed to remain on the tree for 48 hours, when the tannin was determined. In the latter case the tannin was about three times as great as in the former. Apples which had fallen from the tree were also analyzed for tannin and showed about twice as much as in the case where the enzyme action was stopped if such was the cause of the action. However, it was thought that this action should be traced even further than shown in the above preliminary experiments and the first and best method that suggested itself (Thompson) was to follow the action by tracing the soluble nitrogen in content which would decrease if tannin or a tannin-like body was formed which would unite with the proteid bodies in the fruit juices. Accordingly, juices were prepared from a number of different fruits and substances where such action might occur. The materials used were green walnut hulls, ripe apples, green apples and pears; they were first ground through an ordinary meat chopper and pressed through canton flannel. These juices were sampled immediately after pressing out and every 24 hours thereafter, until fermentation was apparent, the samples being filtered through asbestos by suction, and the soluble nitrogen determined with the following results:[2]

Ripe apples—no decrease.
Green apples—64 per cent. decrease in 48 hours.
Pears—14 per cent. decrease in 48 hours.
Walnut hulls—16 per cent. decrease in 94 hours.

To further prove that the action might be due to enzyme action one sample of the above walnut juice was brought to boiling temperature and kept there 30 minutes. In this case for the first 48 hours there was practically no decrease in the soluble nitrogen, but after 94 hours it showed about 6 per cent.

In these experiments it will be noted that there was positive proof of the formation of a tannin-like body that had the power of precipitating proteid matter, thus causing part of the nitrogen to be precipitated in the insoluble form, but as yet the nature of the enzyme was not determined further than this property, although it had been previously shown that the juices had the power of decomposing hydrogen peroxide.

Therefore, a similar series of experiments were carried out by grinding the fruit with calcium carbonate, as suggested by Appleman's work on catalase, in which he showed that the activity of the catalase could be preserved by such treatment. However, when the juices were filtered off and the soluble nitrogen determined, there was no decrease in any case, but fermentation set up in about 36 hours, which was a considerably shorter time than in the previous experiments.

On allowing the calcium carbonate precipitate to settle and testing both the precipitate and the supernatant liquid with H_2O_2 by the method given by Appleman, it was shown that all of the catalase was carried down by the

[2] Complete data in "The Preparation and Properties of an Oxidase Occurring in Fruits," by H. P. Bassett and Firman Thompson, *Journ. Amer. Chem. Soc.*, 33: 416–423, 1911.

calcium carbonate and none whatever remained in the supernatant liquid. Accordingly, this precipitate was filtered off on a Buchner funnel and allowed to dry over sulphuric acid.

It is well known that tannin as such can not exist to any considerable extent in the presence of proteid matter, since these two substances form a precipitate, but as it has been shown repeatedly by the ferric chloride and similar tests that a body existed in the plant cells which gave these tests, the conclusion was drawn that this body must be poly-atomic-phenol that would not precipitate proteid matter. Accordingly, gallic acid was selected for our following experiments.

Therefore, a quintuple set of experiments were then carried on, one set with tannin, a second set with gallic acid, a third set with gallic acid plus enzyme, a fourth set with sodium gallate, and a fifth set with sodium gallate plus enzyme. The experiments were made by putting about 33 c.c. of liquid medium in each of a number of 200 c.c. Erlenmeyer flasks. The tannin, gallic acid and sodium gallate were added to the medium in the following proportions: .025, .05, .1, .2, .4, .6, .8 and 1 per cent. The amount of enzyme was constant throughout the two series in which it was used.

These experiments showed that the organism (*Cunninghamiella echinulata*) used made its best growth in the check and in the gallic acid, and the next best growth in the sodium gallate. The gallic acid plus the enzyme, the sodium gallate plus the enzyme, and the tannic acid all showed a tendency to check the growth in the lower percentages and to completely inhibit it in the higher percentages. However, the results with the next organism (*Glomerella rufomaculans*) were radically different and led to further investigations. In preparing the enzyme with calcium carbonate, as suggested by Appleman's work on catalase, it has been shown above that the precipitated calcium carbonate carried down the catalase completely, but upon testing the supernatant liquid with guiacum it was shown that the presence of an oxidizing enzyme still existed in solution. However, upon testing the precipitate with guiacum after dissolving out the calcium carbonate and acidifying, with acetic acid, it also showed that a considerable portion of this oxidizing enzyme was carried down by the calcium carbonate, and upon drying this precipitate it was shown to absolutely lose its activity. Thus in drying the catalase precipitated by calcium carbonate the portion of the oxidizing enzyme carried down with it was killed, thus explaining the marked difference, as shown above. It should be stated here that in the first experiment referred to above the calcium carbonate precipitate was not completely dry, while in the second it was quite dry. Thus in the first case a considerable amount of the oxidizing enzyme still existed and exerted its influence on the transformation of the gallic acid into the tannin-like body, while in the second case the oxidizing enzyme had been destroyed by the drying and no such action took place to any considerable extent.

It was now evident that we had two enzymes instead of one, and that they could be completely separated from each other by their properties, as stated above, but in such a large proportion of the oxidizing enzyme carried down by the calcium carbonate precipitate it became evident that methods would have to be devised for obtaining the oxidizing enzyme from the supernatant liquid. Accordingly, the supernatant liquid was drawn off and treated by the general method for enzyme precipitation, namely precipitation with alcohol (60 per cent.). This precipitate carried practically all the enzyme down with it. This was allowed to settle and finally collected on a Buchner funnel. As it had been shown that it could not be dried, it was now prepared for use by suspension in water, and in this manner used in the following experiments. The ferric chloride and other tests on the plant cell contents showed the presence of a poly-atomic phenol, and it was evident that the formation of the tannin-like body from the poly-atomic-phenol could probably be carried out in artificial solutions. However, it has been previously shown by Bertrand in work-

ing with laccase prepared from the sap of the lac-tree, and Lindet with an oxidase found in cider and wines, that these enzymes possess the property of oxidizing certain poly-atomic-phenols; *e. g.*, hydroquinone to quinone and pyrogallol to purpurogallic. Thus the following experiments were planned to study this property of the enzyme by preparing artificial solutions of gallic acid and albumen and measuring the rate and extent of the formation of the tannin-like bodies by the decrease of the soluble nitrogen.

Accordingly, 500 c.c. quantities of the following solution were prepared:

No. 1—gallic acid alone, 4 per cent.
No. 2—gallic acid and enzyme.
No. 3—gallic acid and enzyme.
No. 4—gallic acid, albumen and enzyme.
No. 5—gallic acid, enzyme and albumen.
No. 6—enzyme alone.

The albumen used was a solution of egg white which had been filtered through absorbent cotton and contained 1.36 grams of nitrogen per liter. The enzyme was prepared from pear juice by grinding fruit in calcium carbonate, pressing out the juice and allowing it to settle, drawing off the supernatant liquid and precipitating the enzyme with 60 per cent. alcohol, collecting on Buchner funnel, and suspending this precipitate in distilled water. Fifty cubic centimeters of this suspension was used in each case. A series of experiments similar in every particular except 50 c.c. of 3 per cent. hydrogen peroxide was added in each case, and was carried out in the same time. In about an hour after the enzyme had been added a very heavy flocculent precipitate had formed and settled, in those flasks containing all three constituents, viz., gallic acid, albumen and enzyme. Those containing gallic acid and enzyme without albumen had turned a rich wine red color, presumably from the oxidization of the gallic acid.

Samples of 50 c.c. each were taken after 15 hours, and every 24 hours thereafter until there was no longer any decrease in the nitrogen or until the solution showed signs of fermentation.

No. 2, containing gallic acid and albumen, showed no change in soluble nitrogen after 5 days.

No. 4, containing gallic acid, albumen and enzyme, showed a marked decrease in soluble nitrogen amounting to 70 per cent. in five and one half days, 26 per cent. being precipitate in the first 15 hours.

The corresponding solution containing hydrogen peroxide showed a similar decrease, but was considerably more rapid, amounting to 47 per cent. in the first 15 hours.

Having made this further investigation, as outlined above, thus determining some of the properties of this oxidizing enzyme and at the same time explaining our former results, it was now desirable to try the effects of this pure enzyme preparation upon certain fungi. Therefore, *Cunninghamiella echinulata, Glomerella rufomaculans, Pestalozzia breviseta* and *Penicillium aureum* were used in a quintuple set of experiments according to the method previously referred to, but using the pure oxidase instead of the calcium carbonate precipitate. In all cases there was decided action increasing with the increased amount of gallic acid or sodium gallate used.

Since the above experiment showed such high toxic effects which could not all be accounted for by the fermentation of the tannin-like body, it was decided to carry out further experiments on the germicidal properties of the solutions formed. It had also been previously noted that the juices that had been prepared by grinding with calcium carbonate, and in which there was apparently no action of the enzymes fermented much more readily than the juices that had been prepared by grinding without calcium carbonate, and it was evident that the other bodies formed in the reaction might have some germicidal properties. Accordingly, six days after the experiment with gallic acid to show that the precipitate of the soluble nitrogen had been started, plate cultures on agar-agar and gelatine were made from the solution containing gallic acid and albumen; gallic acid, enzyme and albumen; enzyme and albumen. Cultures in beef tea were also made at the same time.

Ninety-six hours after the setting of the cultures the following results were noted. In both cases where the gallic acid and enzyme were not present together there was a heavy fungus growth; but in both cases where they were present together there was a very slight growth in all media. Thus the conclusion would be that the body formed by the action of the enzyme and gallic acid had a marked inhibitive effect on fungus and bacterial growth. It was apparent that for these germicidal effects to be of any value to the fruit it would necessarily have to have quite a rapid reaction in order to keep out any chance of infection, and also from the almost instantaneous appearance of the precipitate; and from the data obtained for the transformation of the soluble nitrogen it was inferred that the action was comparatively rapid, and accordingly an experiment was planned to obtain further data on this point. A solution consisting of 100 c.c. of albumen solution, 200 c.c. of 1 per cent. gallic-acid solution, 50 c.c. of enzyme suspension, and 50 c.c. of 3 per cent. hydrogen peroxide was prepared and diluted to 500 c.c. Another solution which was the same in every respect with the exception of the hydrogen peroxide, which was omitted, was prepared at the same time. Samples of these two solutions were taken every fifteen minutes, for about two hours, and the soluble nitrogen determined. The solution could not be obtained clear on filtering and no flocculent precipitate separated out as in the previous cases. The determinations of nitrogen also showed no decrease in the soluble nitrogen. Thus it was apparent that for some reason the action was not taking place as before, but on adding 60 c.c. more of the enzyme suspension in each case, a heavy flocculent precipitate immediately formed and settled rapidly, and the first sample was taken ten minutes later, filtered as rapidly as possible, and the soluble nitrogen determined showed a decrease of 42 per cent. in the solution containing the hydrogen peroxide and 53 per cent. in the other.

The same experiment was then repeated with the constituents in the same proportions, but the quantity of the enzyme suspension was increased to 150 c.c. The flocculent precipitate appeared at once and settled immediately. Samples were taken every 15 minutes for the first hour, and at longer intervals thereafter for 4½ hours. The decrease in the soluble nitrogen amounted to about 30 per cent. in the first 15 minutes in the solution containing the hydrogen peroxide, and about 23 per cent. in the other. There was, however, practically no further change in the soluble nitrogen, up to four and one half hours, when the sampling was discontinued.

These results no doubt show conclusively that the action carried out by the enzyme is very rapid, but will take place only when the concentration is above a certain undetermined minimum, which point is of very great importance when acting as a protective agent for the fruit.

Analysis of the fruits (apples and pears) made throughout the season, where identical conditions were adhered to, showed a gradual decrease in tannin content. It is well known that fungus parasites increase in activity throughout the season and are most destructive as the fruits approach maturity. Later in the season tests were made for the determination of the localization of the enzyme in the fruit by the use of the guiacum solution, which showed that in the case of pears the blue color developed first around the core and immediately under the peel, but finally developed uniformly over the freshly cut surface. As cold weather approached, the pears were removed from the trees and stored in a cool, dry place, by which means it was hoped that the work might be continued for some time. An attempt to prepare some of the enzyme from these pears eight days after their removal from the tree resulted in a preparation which had lost practically all its power. On testing the freshly cut surface with guiacum solution no blue color was developed, excepting rather faintly immediately around the core. The supply of pears having been exhausted, apples were examined in the same manner from time to time, and showed a gradual decrease in the amount of enzyme

until to date (February 8, 1911), showing only a slight trace around the inner part of the core.

It is interesting to note that early in the season several normal fruits were injured by passing a sharp instrument through them from side to side and allowing them to remain on the tree for 48 hours thereafter. A section was then made through the injury and the guiacum solution applied. The blue color developed first quite strongly around the walls of the injury, followed gradually by the other parts of the pear.

From the preceding it will be readily seen that there exists in the normal living fruit two enzymes, a catalase and an oxidase. The latter is probably most abundant in the early part of the season, gradually decreasing in activity as the fruit approaches maturity and ripens. Furthermore, from the above results it appears that tannin as such does not exist in any part of the normal, uninjured fruit previous to maturity, except possibly a small amount in the peel, but exists as a poly-atomic phenol, which upon injury is acted upon by the oxidase and forms a tannin or tannin-like body having the property of precipitating proteid matter, and at the same time forming a germicidal fluid. This oxidase acts only in an acid solution, and when present in an amount above a certain undetermined minimum. The above conditions are always present in normal immature pomaceous fruits. When normal, immature fruits are subjected to injury by fungi, insects, or mechanical agencies, the action of the oxidase on poly-atomic-phenol is brought about with the effects as stated above.

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THE RELATION OF PERMEABILITY CHANGE TO CLEAVAGE, IN THE FROG'S EGG

UNFERTILIZED eggs (taken from the uterus) of the wood frog, *Rana sylvatica,* were caused to assume the normal orientation in the jelly, and to segment, by electrical stimulation. An alternating current of 60 cycles and 110 volts was passed through the tap water containing the eggs, from platinum electrodes about two inches apart. Stimulation for one second seemed to give the best results. The eggs were placed in fresh water immediately after stimulation.

Similar eggs were caused to segment by mechanical stimulation, even while the jelly remained intact. However, the most reliable mechanical means of inducing cleavage was found to be Bataillon's method of pricking the egg with an extremely fine needle. The first cleavage furrow often passed through the point of puncture.

Thousands of eggs were operated on. Control eggs were kept to both sets of experiments, and showed no segmentation or rotation within the jelly.

The following indirect evidence is given to show that a change in permeability is associated with both of these means of inducing cleavage:

1. These "stimuli," if applied in greater intensity or duration than is necessary to produce cleavage, result in rapid osmotic exchange with the medium and death of the egg.

2. Similar electrical and mechanical "stimuli" produce segmentation in the sea-urchin's egg, a process which I have shown to be preceded by an increase in permeability.

With the exception of the rate of oxidation, this change in permeability is the only known common intermediate step between fertilization or artificial "stimulation," on the one hand, and cleavage on the other. Furthermore, there is indirect evidence to show that increase in permeability is associated with fertilization, in the frog's egg, as I have shown to be the case in the sea-urchin's egg: Backman and Runnström[1] observed that, whereas the osmotic pressure (freezing point lowering) of the ripe ovarian egg of the frog is the same as that of frog's serum, the osmotic pressure of the fertilized egg is the same as that of the pond water in which it lies. Since the frog's

[1] *Biochem. Zeitschr.*, 1909, XXII., 390.